# Safetygram

NCI-Frederick

ISM-193 Laboratory Personnel AUGUST 2004

## Work with Adenovirus and Adenoviral vectors

### General

Adenoviruses are a frequent cause of acute upper respiratory tract (URT) infections, i.e. "colds". In addition, they also cause a number of other types of infection. Droplets, fomites and close personal contact are the most common means by which adenoviruses can spread from person to person. In most healthy adult humans infections with adenoviruses are self-limiting however in immunosuppressed hosts, they can cause serious morbidity and increased mortality. Adenovirus was first isolated in 1953 by investigators trying to establish cell-lines from adenoidal tissue of children removed during tonsillectomy and from military recruits with febrile illness. Adenoviruses can infect a wide variety of cell types and tissues in both dividing and non-dividing cells. This characteristic, together with their relative ease of preparation and purification, has led to their extensive use as gene vectors.

### Viral Vector

Adenoviruses are non-enveloped virus with a double stranded DNA genome of approximately 36kb. Adenoviral vectors are usually based on attenuated or less pathogenic strains that are typically rendered replication incompetent through the deletion of one or more genes or gene regions. The vectors are rendered replication incompetent through the E1(a) (early gene) or E2, E3, and E4 (late genes). During production of the vector, cultured cells that express the gene product will supply the missing gene components. An example of this would be HEK 293 or 911 cell lines which are E1(a) complementing cell lines. Use of an Adenoviral vector to deliver a transgene has a number of advantages:

1. Does not integrate into the host's chromosome
2. Infects a broad range of cells (dividing and quiescent cells)
3. Able to accept large amounts of foreign DNA
4. Produced easily at high titers
5. Achieve a high level of expression

### Precautions

1. Adenovirus is a pathogen of respiratory and gastrointestinal mucous and eye membranes, and does not have to be replication-competent to cause corneal and conjunctival damage. Goggles should be worn when working with the agent/vector.

2. The replication-defective virus may be complemented in vivo thereby causing the vector to become replication competent.

3. Adenovirus (unlike HIV or herpes) is quite stable. After having been extracted with ether, and/or chloroform, it can still be infective.

4. Signs and labels must be placed to indicate each area where Adenovirus is used or stored (including Biosafety cabinets, incubators, refrigerators, laboratory entrance doors, etc.).

## Laboratory Practices

1. All vacuum lines must be fitted with a HEPA filter (an example is the "Vacushield ™" in-line hydrophobic filter, Product # 4402 from Gelman Science).

2. No work with Adenovirus is permitted on the open bench. A Biosafety Cabinet must be used for all manipulations including (but not limited to):
   a. pipetting
   b. harvesting infected cells for RNA
   c. loading and opening containers

3. Centrifugation must be done in closed containers using sealed rotors.

## Animal Use

1. When animals are infected with Adenovirus/Adenoviral vectors, an Animal BSL-2 area must be approved and used for the procedure. Concurrent approvals are needed from the Institutional Biosafety Committee (IBC) and the Institutional Animal Care and Use Committee (IACUC).

2. All necropsy must be performed in a necropsy room using Animal BSL-2 + Adenovirus precautionary practices and procedures.

3. Infected animals may excrete Adenovirus (especially in the first 72 hours after infection). Precautions must be taken not to create aerosols when emptying animal waste material and when washing down cages, or cleaning the room with pressure hoses.

4. It is recommended by the EHS that the lab personnel or animal husbandry technicians, specifically trained on the handling of adenovirus infected animals, be responsible for all animal husbandry practices during the first 72 hours following infection of the animal.

5. Special training must be given to all animal husbandry personnel involved on an Adenovirus experiment. Training should cover the hazards associated with the work, required practices and procedures and proper handling of bedding, cage washing, and all other husbandry materials associated with the experiment.

## Employee Exposure

1. Eye Exposure from splash or aerosols - rinse a minimum of 15 minutes in eye wash or flush area with water and report to OHS immediately after flush. Follow NCI-Frederick Exposure Control Plan procedure for reporting occupational exposures to potentially infectious material. Dial 911 after-hours to report exposure and obtain assistance.

2. Needlestick and/or non-intact skin exposure – Contaminated skin should be scrubbed for ~20 minutes using a 10% povidone iodine solution (such as Betadine) and copious amounts of water. Report to OHS immediately after scrub. If the exposure occurs during off-hours contact the 911 emergency number. Follow NCI-Frederick Exposure Control Plan.

3. SYMPTOMATOLOGY: Acute Respiratory Illness (cold-like symptoms); pneumonia. Conjunctival infection (or red eye), corneal inflammation including possible scarification.

**Personal Protection Equipment**

1. Gloves (nitrile, latex, etc)

2. Wrap around outer clothing when introducing vector into animals or performing necropsies. Labcoats are adequate for tissue culture manipulations.

3. Goggles (not to be confused with safety glasses).

4. N-95 or HEPA Respirator, to be used with concentrated titers and highly aerosolizing procedures outside of the Biological Safety Cabinet (contact EHS for information on the Respiratory Protection Program).

## Decontamination

1. The most effective germicides (with a minimum 15 min. contact time) are: (this is not to be performed for personnel exposure!)

    - Phenol (5%)
    - Sodium hypochlorite 0.5% solution recommended (*household bleach diluted to 200 ppm is the minimum concentration for virucidal activity of Adeno Type 2)

    * 2001, H. Prince & D. L. Prince, Principles of Viral Control and Transmission, in Disinfection, Sterilization, and Preservation, 4th ed. p. 545